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Lonely No More: *p53* Finds Its Kin in a Tumor Suppressor Haven

Minireview

Moshe Oren
Department of Molecular Cell Biology
The Weizmann Institute of Science
Rehovot 76100
Israel

Oncogenes and tumor suppressor genes often come in families. c-*myc* has N-*myc* and L-*myc* as cousins, H-*ras* has K-*ras* and N-*ras*, and *Rb* has *p107* and *p130*. It was thus only logical to expect the same for *p53*, a pivotal tumor suppressor gene which is the most frequent target for genetic alterations in human cancer (for recent reviews, see Gottlieb and Oren, 1996; Ko and Prives, 1996; Oren and Prives, 1996; Levine, 1997). However, *p53* repeatedly defied these expectations: all attempts to find *53*-related genes, by low stringency hybridization or by degenerate PCR techniques, came up empty-handed. This has led to the firm conviction that a *p53* gene family does not exist.

This conviction—perhaps one of the few that was agreed upon by the p53 field—is no longer correct. A novel gene has now been described whose products display striking structural and functional resemblance to those of *p53* (Kaghad et al., 1997). Moreover this gene, termed *p73* (big brother of *p53*?), may also be a tumor suppressor gene, perhaps one long sought-after in neuroblastoma and other cancers.

p73 started off as an ugly duckling—a false positive cDNA clone in a screen for mediators of insulin signaling (Kaghad et al., 1997). However, as soon as its sequence was obtained, its swan-like charm emerged.

p73—A Structural and Functional Homolog of p53

The last decade has yielded extensive information on p53. Biologically, the best known activities of p53 are cell growth arrest and induction of apoptosis. Both probably require activation of latent p53 by incoming signals, often coupled with a substantial increase in overall cellular p53 levels.

Human p53 comprises 393 amino acid residues (Figure 1). It includes three main functional domains: an N-terminal acidic transactivation domain (TAD), a central DNA-binding core domain (DBD), and a C-terminal homo-oligomerization domain (OD). All three domains are required for efficient binding of p53 to recognition sites within its physiological target genes and for transcriptional activation of these genes.

Five evolutionarily conserved boxes (I to V in Figure 1) were identified through comparison of p53 sequences from various vertebrates. Four of these are in the central DNA-binding core domain. Importantly, the vast majority of tumor-associated p53 missense mutations occur within this core domain, often in boxes II to V. Such mutant proteins usually fail to trigger transcription of p53 target genes, resulting in loss of tumor suppressor activity.

The newly discovered human *p73* gene encodes two distinct polypeptides (Figure 1). The longer one, denoted p73α, comprises 636 residues. The shorter form, denoted p73β, arises through alternative splicing of the *p73* transcript (Kaghad et al., 1997) and contains only 499 residues. With the exception of the last five residues, which are unique to p73β, the rest of the protein is identical to the corresponding region in p73α. Even though the two forms appear to differ in some properties (see below), there is no indication so far that their expression is differentially regulated (Kaghad et al., 1997).

What do we learn from the p73 protein sequence? Remarkably, the conservation of the core DNA-binding domain shows a 63% identity with p53 (Figure 1). There is also a striking conservation of critical residues which, in p53, either interact directly with target DNA or are required for the proper folding of the entire domain (Kaghad et al., 1997). The pivotal role of these particular residues is highlighted not only by direct structural studies (Cho et al., 1994), but also by the fact that they are major hot spots for mutation in human cancer. Based on this striking similarity, one might predict that not only will p73 bind to DNA, but it may recognize DNA targets very similar to those of p53.

Significant conservation also exists within the other two main functional domains. This is less pronounced within the N-terminal transactivation domain. However, one should recall that the amino acid sequence of the latter is rather poorly conserved even between human and murine p53; what is conserved between species, the overall acidic nature of the transactivation domain, is also maintained in p73.

All the above suggest that p73, like p53, is a sequence-specific transactivator, which probably requires oligomerization, and is likely to regulate genes that at least partially overlap those targeted by p53. Strong support exists for some, though presently not all, of these predictions. Analysis by the yeast two-hybrid assay revealed that p73β can homo-oligomerize as efficiently as p53 (Kaghad et al., 1997). Surprisingly, perhaps, p73α failed to form homotypic interactions. Moreover, the experiments suggest a significant, albeit relatively weak, interaction between p73β and p53. It will be interesting to see whether mixed oligomers indeed exist in cells, and how this affects the biochemical and biological activities of p53.

More importantly, p73 overexpression can trigger at least one canonical p53 target gene, $p21^{Waf1}$. The $p21^{Waf1}$ gene is strongly induced by p53 in many cell types (El-Deiry et al., 1993). The p21 protein is a potent inhibitor of cyclin-dependent protein kinases—the driving motors of the cell cycle—and augmented levels of p21 halt cell cycle progression. It is thus not surprising that p21 is a key effector, though not the only one, of p53-mediated growth arrest (Figure 2).

As shown now, p73 overexpression also induces p21 (Kaghad et al., 1997; Table 1). Cells transiently transfected with a p73α expression plasmid display markedly elevated levels of p21, comparable to those obtained with wild-type p53. Importantly, p21 induction is abolished when a single Arg residue at position 292 of p73 is mutated to His; this residue is analogous to Arg 273 of human p53, which is often mutated in human cancer and whose conversion to His abrogates the ability of p53

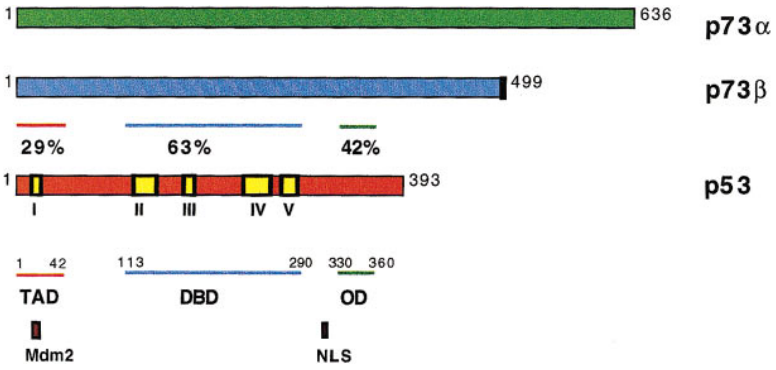

Figure 1. Comparison between the Human p73 and p53 Proteins

p73α and p73β denote two forms derived through alternative splicing. The black bar at the C-terminal end of p73β identifies a stretch of five residues not shared with p73α. The five evolutionarily conserved blocks in p53 are labeled I to V. The positions of the Mdm2 binding motif and the nuclear localization signal (NLS) of p53 are also indicated, as well as the following p53 protein domains: TAD, transactivation domain, residues 1–42; DBD, DNA-binding domain, residues 113–290; OD, oligomerization domain, residues 330–360. The percentage amino acid sequence identity between p53 and p73 within each domain is indicated.

to activate relevant target genes. Hence, p73 appears to duplicate faithfully the transcriptional function of p53 on at least one critical target gene.

Given the centrality of p21 in the p53 pathway (Figure 2), it was not surprising that, like p53, p73 is also capable of blocking cell proliferation when overexpressed. In a standard stable transfection assay, p73 effectively blocked colony formation (Kaghad et al., 1997; Table 1). This can be taken as preliminary evidence that p73 has attributes of a tumor suppressor, at least in this in vitro assay.

Induction of apoptosis by p53 is less well understood. Most probably, it also involves activities of p53 distinct from transactivation of specific target genes (Gottlieb and Oren, 1996; Ko and Prives, 1996; Levine, 1997). At present, it is impossible to tell whether p73 also possesses such activities (Table 1). Nevertheless, transcriptional activation does play an important part in p53-mediated apoptosis; genes that may serve as downstream effectors have been identified (Figure 2). It is conceivable that the ability of p73 to up-regulate p53 target genes is not limited to *p21*. Obvious questions are whether p73 can up-regulate apoptosis-related genes such as *bax* and *Fas/Apo1*, and whether p73 overexpression can cause apoptosis.

In summary, p73 looks very much like p53, and may also work very much like p53. However, given the limited amount of available data, much more needs to be done before the extent of overlap between the two can be critically evaluated.

p73—*A Long Sought-after Tumor Suppressor Gene?*

The similarity to *p53* provided one provocative clue that *p73* may be a new tumor suppressor gene. The other clue came when the *p73* gene was mapped to the subtelomeric p36 region of human chromosome 1 (Kaghad et al., 1997). Deletions near the tip of the short arm of chromosome 1, spanning 1p36, are common in a variety of human tumors including neuroblastoma, colon cancer, melanoma, and breast cancer (Schwab et al., 1996). This observation has spurred an intensive search for tumor suppressor genes in this chromosomal region. Recent studies suggest that this region contains at least two, and perhaps more, distinct tumor suppressor genes (Versteeg et al., 1995; Schwab et al., 1996). One putative tumor suppressor gene appears to be selectively deleted and presumably inactivated in neuroblastomas that harbor amplification of the N-*myc* protooncogene (Caron et al., 1995). Such tumors typically display large deletions in 1p; the critical tumor suppressor gene appears to reside in region 1p35–36.1, proximal to the position of *p73*. Even more interesting, however, is a second putative tumor suppressor gene, identified in neuroblastoma tumors lacking N-*myc* amplification. It has been tentatively mapped to 1p36.2–3, within an 8 megabase (Mb) region. Importantly, *p73* also resides in this same region. A particular feature of this putative tumor suppressor gene is that it appears to be imprinted. Thus, in tumors retaining only one copy of the corresponding chromosomal region, this copy is almost always derived from the paternal chromosome. This

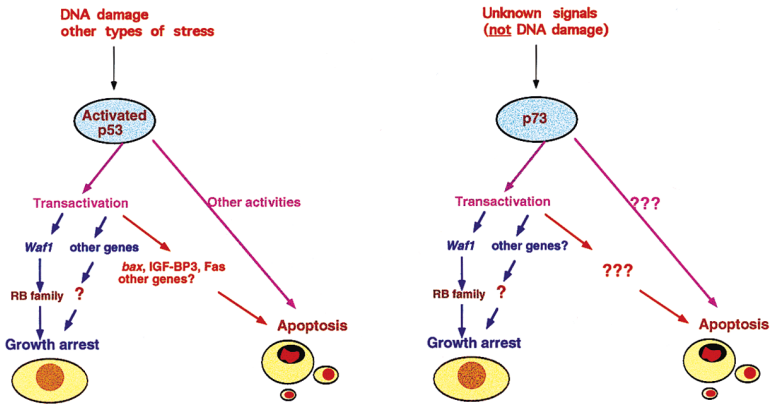

Figure 2. The p53 Pathway and the Putative p73 Pathway

For a more detailed description, see Oren and Prives, 1996.

Table 1. Comparison of Reported Features of p53 and p73

Activity	p53	p73
Activation of $p21^{Waf1}$	yes	yes
Location (active form)	nuclear	nuclear
Growth arrest	yes	yes
Apoptosis	yes	?
LOH[a] in cancer	many cancers	neuroblastoma
Mutations in cancer	very frequent	none yet
Germline mutations	Li-Fraumeni syndrome	none yet
Down-regulation by Mdm2	yes	?

[a]LOH, loss of heterozygosity.

strongly suggests that the paternal locus is imprinted and consequently transcriptionally silent. Thus, the loss of only the maternal copy of the gene would be sufficient to practically render the cells negative for the corresponding transcript and protein. Any serious contender for the candidate gene should therefore first be found to be imprinted, and additionally be silent in tumor cells retaining only one allele.

At least at a first approximation, *p73* appears to fulfill these requirements. First, there is clear evidence for monoallelic expression, as is expected of an imprinted gene. A normally occurring polymorphism has been identified in the noncoding region of the *p73* gene (Kaghad et al., 1997). Using this polymorphism, *p73* mRNA was analyzed in the peripheral blood cells of five heterozygous healthy individuals; in all cases, only one allele was found to be expressed, suggesting that the other one was imprinted. Moreover, in the single case so far where the origin of the *p73* mRNA in normal cells could be determined, it was derived predominantly if not exclusively from the maternal allele. Hence, *p73* appears to be paternally imprinted, exactly as predicted for the putative 1p tumor suppressor gene. Needless to say, one has to be extremely reserved about information coming from such a small number of samples, regarding it mainly as an incentive for obtaining more complete, unequivocal data.

Analysis of tumor-derived cell lines also largely agrees with the notion that *p73* is a strong candidate for the "real" 1p tumor suppressor gene. In particular, extremely low levels of *p73* mRNA were found in the majority (4/6) of neuroblastoma cell lines harboring chromosome 1p deletions (Kaghad et al., 1997). This stands in stark contrast with lines derived from some other tumors and from neuroblastoma without a deletion in 1p, where easily detectable *p73* expression was much more common. Hence, the *p73* allele retained in these four neuroblastoma lines may indeed be imprinted. Moreover, in one of the two 1p deletion-positive neuroblastoma lines that retained *p73* mRNA, no p73 protein was detectable.

Nevertheless, the picture is not perfect. The main discomforting observation is that despite extensive efforts, no mutations could be found within the remaining *p73* allele in tumor cells with 1p deletions. One might argue that there is no real selective pressure for mutating *p73* if only the imprinted, nonexpressed allele is retained. However, *p73* is also wild-type in the one neuroblastoma line that expresses the protein abundantly. Clearly, if one wishes to establish that *p73* is a true tumor suppressor gene, these critical issues will need to be resolved more rigorously. Until that time, *p73* should simply be regarded as a good candidate.

New Answers (Perhaps) to Old Questions, and New Questions to Be Answered

The discovery of p73 sheds new light on a number of well-known facts about p53. One of the most obvious is the almost normal development of *p53* knock-out mice (Donehower et al., 1992). Given the numerous reports about the role of p53 in differentiation, apoptosis, and proliferation of cultured cells, the lack of dramatic developmental phenotypes was rather disappointing. Although some defects were later uncovered, they were limited in scope and penetrance. With other key regulatory proteins, mild phenotypes of single knock-outs were explained by functional redundancy with other family members. However, such explanation could not be offered for p53. Not until p73, that is. It may now be proposed that p73 doubles up for p53, and only fails to do so under conditions of genomic damage, where a specific function of p53 becomes irreplaceable. Of note, the main difference so far between p53 and p73 is that the latter protein is neither stabilized nor activated by DNA damage (Kaghad et al., 1997; see Figure 2). Hence, it may not play a role in monitoring genomic damage. As to the possibility that p73 substitutes for developmental functions of p53, the answer is likely to come from crosses between *p53* and *p73* knock-outs. On a cautionary note, now that p73 has been identified, there is no compelling reason to exclude the existence of additional p53 family members. Such yet-to-be-uncovered genes are likely to complicate the genetic analysis.

Another observation that *p73* may explain is that, unlike most tumors, neuroblastomas very rarely carry *p53* mutations (Ohgaki et al., 1993). It could now be proposed that in the particular normal cells that give rise to neuroblastoma it is p73, rather than p53, that performs the crucial tumor suppressor job. Hence, once p73 is inactivated, there will be no significant selective pressure to inactivate p53 as well. The actual picture is, however, more complex. While the *p53* gene remains wild-type in almost all neuroblastomas, the protein may be misplaced: rather than being nuclear, it accumulates in the cytoplasm (Ostermeyer et al., 1996). It is presently controversial whether this is truly a functional inactivation of p53, which blocks its ability to respond to stress and maintain genomic integrity. Yet, if this cytoplasmic sequestration truly results in p53 incapacitation, it may make the need to invoke p73 less obvious. Even so, one could still propose that it is the lack of functional p73 protein that underlies the cytoplasmic retention of p53. Future work should clarify this issue.

It is thus yet to be proven that *p73* is truly the long sought-after 1p tumor suppressor gene. In particular, one worries about the inability to find coding region mutations in cell lines tested to date. This concern is strengthened by the knowledge that single point mutations, analogous to those observed in p53 in human cancer, indeed suffice to abrogate the antiproliferative effect of p73 (Kaghad et al., 1997). Given the high frequency of such mutations in p53, it is puzzling that the same does not pertain to p73 (Table 1). While the

imprinted nature of the *p73* locus offers a reasonable explanation, it is equally possible that *p73* just happens to reside within a chromosomal region harboring a cluster of imprinted genes, and the real tumor suppressor gene may actually be one of *p73*'s neighbors. To resolve this important issue, one may either need to eventually find some tumors, not necessarily neuroblastoma, that bear inactivating mutations in *p73*, or else go systematically through the entire 8 Mb region and rule out all neighboring genes one by one.

In its relatively short history, p53 research has already seen a number of surprising twists. The discovery of p73 may be another major turning point along this road. Judging from past experience, it is quite safe to predict that p73 will soon become the latest spin-off from p53 to develop into a "hot" topic in its own right.

Selected Readings

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